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Fetal haemoglobin, blood transfusion, and retinopathy of prematurity in very preterm infants: A pilot prospective cohort study

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Abstract

Purpose: To identify if there is an association between fetal haemoglobin (HbF) concentration and retinopathy of prematurity (ROP) in very preterm infants.

Methods: Prospective cohort study. Infants born <32 weeks' gestational age or <1501g in two tertiary neonatal units between January 2012 and May 2013 (n=42) were enrolled. Fetal haemoglobin (HbF) and adult haemoglobin (HbA) concentrations were measured using High Pressure Liquid Chromatography from blood samples sent as part of routine neonatal care once routinely requested laboratory tests had been performed. Clinical data were obtained from case notes. We calculated Odds Ratios (95% CIs) to quantify the relationship between initial and mean %HbF with ROP severity (none, Stages 1,2,3).

Results: 42 infants were recruited: mean gestation 28.0w (SD 1.91); mean birthweight 1042g (SD 264). 6 infants died before ROP screening; 14/36 developed ROP (39%) and 22/36 (61%) did not. Infants who developed ROP had similar initial %HbF (83.3% vs. 92.3%, p=0.06) but significantly lower mean %HbF (61.75% vs. 91.9%, p=0.0001) during their inpatient stay than those who did not develop ROP. In Ordinal Logistic regression models adjusted for birthweight, gestation and transfusion volume, mean postnatal %HbF was negatively associated with ROP severity: adjusted OR 0.94 (0.90-0.99) whilst initial %HbF at birth was not: adjusted OR 1.05 (0.97 – 1.16).

Conclusion: Replacing HbF by HbA during transfusion may promote ROP development by rapidly increasing oxygen availability to the retina. Conversely, maintaining a higher %HbF may be a protective factor against ROP.

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Keywords: Retinopathy, prematurity, transfusion, fetal haemoglobin

Introduction

Studies in preterm neonates have highlighted that a balance must be found between the toxic effects of high oxygen saturations and the increased morbidity and mortality associated with targeting lower saturations.[1-4] Birth weight and gestation have also been shown to be consistently independently associated with ROP development. A number of other factors appear to have an association with ROP, including genetics, poor nutrition and poor weight gain, sepsis and necrotising enterocolitis.[5-7] Various studies have identified an association between ROP and blood transfusion.[8-10]

Neonates have a predominance of fetal haemoglobin (HbF) at birth. Approximately 85% of total haemoglobin is HbF in infants born at 35 weeks' gestation which gradually declines until it has disappeared by the age of 1-2 years.[11, 12] HbF has a greater affinity for oxygen compared to HbA, shifting the haemoglobin-oxygen dissociation curve to the left, causing preferential fetal oxygen binding in utero. However, HbF preponderance postnatally in very preterm infants leads to greater difficulty unloading oxygen to the tissues. This is partly offset by a steeper oxygen-haemoglobin dissociation curve than that for HbA, but exacerbated as the levels of 2,3-diphosphoglycerate (a product of glycolysis that promotes oxygen release from oxy-haemoglobin) are low in preterm neonates.[13]

Anaemia of prematurity, in part thought to be related to reduced red cell life span and low erythropoietin levels, is exacerbated by clinical blood sampling, leading to the need for blood transfusion in many very preterm infants.[14] De Halleux V *et al*, 2002,[15] demonstrated clinically that the oxygen-haemoglobin dissociation curve is shifted to the right in preterm infants after blood transfusion. We hypothesised that as the HbF:HbA ratio decreases with blood transfusion, more oxygen is made available to the developing retina for any given arterial partial pressure of oxygen (PaO_2), possibly contributing to ROP development by increasing the oxygen availability in the developing retina and reducing angiogenic drive. We aimed to explore whether there might be an

association between either initial %HbF (on admission after birth) and/or the mean inpatient HbF% (during their hospital admission), with the development of ROP in very preterm infants.

Methods

Study design

We conducted a prospective cohort study across two tertiary neonatal intensive care units in Bristol, UK (St. Michael's and Southmead Hospital). All inborn infants and those retrieved from neighbouring hospitals within 24 hours of birth born <32 weeks' gestation or <1501 grams were eligible for inclusion. Parents were counselled as to the nature of the study and provided with an information leaflet. With informed parental consent all routine EDTA samples taken during the baby's admission were analysed for HbF%, HbA% and HbF:A ratio. No additional blood samples were taken for the purposes of the study. Analysis of HbF and HbA were performed at a single site (Southmead Hospital) using high performance liquid chromatography (BioRad Variant II HPLC analyser; daily calibration; 5 µl blood sample volume). Recruited patients received routine neonatal care and the existing local protocol was followed for blood transfusion thresholds. Infants received on site retinopathy screening by a single consultant ophthalmologist according to national guidelines.[16]

Data collection

Patient case notes were reviewed after discharge from the neonatal unit (discharge home or transfer to other neonatal unit) and demographic and clinical information were extracted in addition to reviewing computerised laboratory reporting systems as follows: gestational age, birth weight, sex, ethnicity, multiple pregnancy, days on respiratory support, days in supplementary oxygen, duration of stay, corrected gestational age at discharge. Haematological data was extracted as follows: Hb, %HbF, %HbA, HbF:HbA ratio, and blood transfusions (date, time and volume as ml/kg). Episodes of infection were identified as definite (blood culture positive, CRP rise >10 and ≥5 days antibiotics),

probable (CRP rise >10 or positive blood culture, and ≥5 days antibiotics), or not present. ROP screening data was recorded from patient notes for the duration of their ROP screening, according to established nomenclature.[17] If there was no ROP at any time, the ROP outcome was recorded as stage 0, whilst for any infant that did develop ROP, the worst stage in the worst eye was used as their outcome. The presence or absence of "Plus" (or "Pre-plus") disease was also noted. For those infants discharged to other neonatal units before retinopathy screening had been completed, data was extracted from BadgerNet, an electronic patient data management system.

Data analysis

Initial %HbF (on admission after birth) and demographics on the cohort were extracted from the notes along with the results from their ROP screening examinations. Mean %HbF (during in-patient stay) was derived using time-weighted averaging (multiplying each %HbF value by the length of time between samples, and then averaging the results for the whole period of an infant's in-patient stay). Initial univariate comparisons were carried out between those infants who developed any ROP versus those who did not, with respect to their HbF values. %HbF from each full blood count was plotted against corrected gestational age. An ordered logistic regression model was used to calculate Odds Ratios (ORs) and 95% Confidence Intervals to estimate the association between the value of the %HbF (either initial, or mean of inpatient stay) with the most severe stage of ROP recorded for each baby (0, 1, 2, 3). Corresponding adjusted ORs were derived after including gestation, birthweight, and total volume of red blood cell transfusions in the logistic regression model.

Analysis was performed using STATA 10 (Stata Corp) and results are presented as number (%), mean (standard deviation), median (inter-quartile range) or OR (95% confidence interval) as appropriate. Ethical approval was obtained from The NRES Committee North East Newcastle and North Tyne 2.

Results

42 infants were recruited between January 2012 and November 2013. No infants were excluded from the study. All parents approached for recruitment during the study agreed to be enrolled except the parents of one set of twins. Demographic data for the cohort, split by ROP status are displayed in Table 1.

Table 1

37 neonates were inborn and 5 infants were outborn. All outborn infants were born in a local district general hospital, retrieved by the tertiary neonatal team and arrived in the tertiary neonatal unit before 10 hours of age. 6 infants died before ROP screening (3 male, 3 female) of which 4 were inborn and 2 were outborn. Infants who developed ROP were more likely than infants who did not develop ROP to be from multiple births ($p=0.027$), more preterm ($p<0.001$), of lower birthweight ($p=0.007$), to have had more transfusions ($p<0.001$), larger volumes infused ($p<0.001$), and to have spent longer on ventilators ($p<0.001$) and Continuous Positive Airway Pressure (CPAP) ($p=0.028$).

24 infants received a transfusion of red blood cells (RBC) during their admission (57%). Of the 36 infants who survived to ROP screening all survived to discharge from the tertiary unit. 22 of these infants did not develop ROP (52% of the initial cohort). 14 infants developed ROP (33% of the initial cohort): stage 1 (n=5), stage 2 (n=4), stage 3 (n=5, 4 of these infants also had plus disease, 4 of these infants received laser treatment and one received intravitreal bevacizumab), grade ≥4 (n=0). No infant developed retinal detachment (Stage 4 or worse) during the study.

Those infants who did not develop ROP had higher initial haemoglobin levels (on admission) than infants who did develop ROP ($p=0.009$), as shown in Table 2 and there was weak evidence that they have a lower initial %HbF (83.3% vs. 92.3%, $p=0.06$). Infants who developed ROP had significantly lower ($p=0.0001$) mean %HbF during their admission compared to those infants who did not develop ROP (Table 2).

Table 2

When plotting HbF% from each full blood count against corrected gestational age there appears to be two populations. Those infants who developed ROP have noticeably lower HbF% when compared to those infants without ROP (Figure 1).

Figure 1

The ordinal regression model produced similar results to the univariate associations above (Table 3).There was only weak evidence that initial HbF% was associated with increasing risk of ROP grade (p=0.070) and this association disappeared after adjusting for birthweight, gestation at birth and volume of transfusion (p=0.261). In contrast there was strong evidence for an association between Mean %HbF and increasing risk of ROP grade in both the unadjusted (p<0.001) and the adjusted analyses (p=0.034).

Table 3

Discussion

Whilst very preterm infants who developed ROP had similar initial %HbF when compared to those who did not develop ROP, we observed that as hypothesized, mean %HbF was significantly lower in the ROP group during their inpatient stay than in the babies who did not develop ROP. A lower %HbF may be a proxy or surrogate marker for sickness, as neonates who are more unwell will be more likely to require and receive blood transfusions. We therefore included volume of blood transfused as an approximate proxy marker of illness severity in the logistic regression model and an association still seemed to hold between lower %HbF and ROP development. There is physiological evidence

supporting left shift of the oxy-haemoglobin dissociation curve with increasing %HbF. It is therefore biologically plausible that a lower HbF concentration (and higher HbA concentration) provides greater oxygen delivery to the developing retina. Evidence of this effect (ROP) has been found in our study. It may therefore be that maintaining higher HbF levels for longer confers some protection against ROP development.

We recognise limitations with our study, notably a small sample size. Identifying causal factors in ROP development is not possible in this small cohort study due to the large number of variables and confounding factors over this time period as well as a relatively heterogeneous patient population. We considered supplemental oxygen therapy to be part of the causal pathway leading to ROP development, rather than as a confounder and did not therefore adjust for it in these models. In a larger study, it would be useful to explore in more detail the relationship between supplemental oxygen, intercurrent illness and blood transfusions on %HbF and on the development of ROP. There is a need for further research to establish evidence for a potential causal relationship between HbF% and ROP risk.

Various interventions including laser therapy and angiogenesis inhibitors are utilised to manage established ROP and much research is ongoing in this area. However, if high risk infants can be recognised and there is a possibility of enhancing intrinsic protective factors, then ROP development could potentially be minimised or prevented. Delayed cord clamping has been shown to deliver additional blood to the newborn from the placental bed.[18, 19] This could potentially reduce or delay the need for subsequent transfusion, in turn facilitating maintenance of HbF. There is already reluctance to transfuse liberally in preterm neonates due to NEC risk, exposure to donors and risk of transfusion reaction, but risk of ROP development might also need to be considered if further research supports the hypothesis that early loss of HbF predisposes an infant to developing ROP.

Conclusions

To our knowledge this is the first study to investigate and find an association between HbF concentration, blood transfusion and ROP development. It is possible that HbF is a protective factor against ROP and that transfusion of adult (HbA) blood may play a part in ROP development by suddenly making more oxygen available to the developing retina and downregulating VEGF, resulting in arrest of the advancing front of retinal vasculature. Subsequent reduction of oxygen supply leads to ischaemia of the unvascularised peripheral retina and marked upregulation of VEGF with the development of fundoscopic signs of ROP. Larger studies are required to investigate these associations further.

Summary

What was known before

- Retinopathy of prematurity (ROP) is a major cause of morbidity in the preterm population
- Oxygen is known to be the predominant causal factor in ROP development
- ROP has been shown to be associated with blood transfusion

What this study adds

- Blood transfusion dramatically reduces the fetal haemoglobin (HbF) concentration
- Those infants who develop ROP have significantly lower mean fetal haemoglobin (HbF) levels

Maintaining higher HbF levels may be protective against ROP

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Contributors

C Stutchfield: Contributed to study design. Recruited patients, designed data extraction proforma and extracted all case note and laboratory data. Assisted with data analysis. Drafted scientific paper.

A Jain: Contributed to study design. Recruited patients, assisted with data analysis, edited scientific paper.

D Odd: Contributed to study design. Recruited patients, statistical analysis, edited scientific paper.

C Williams: Contributed to study design, performed all ROP screening, assisted with data analysis, edited scientific paper.

R Markham: Principal Investigator. Study inception and design. Secured charitable funding and edited scientific paper.

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Tables

Table 1: Demographic and ROP* outcome data

Variable	No ROP	ROP	P
Male	9 (40.9%)	5 (35.7%)	0.441
Non-white ethnicity	2 (9.1%)	0 (0%)	0.411
Multiple birth	11 (50.0%)	1 (7.1%)	0.027
RBC† transfusions	0 (0-1)	3 (1-5)	<0.001
Total RBC transfusion (ml/kg)	0 (0-20)	53 (20-103)	<0.001
Culture positive sepsis	2 (9.1%)	3 (21.4%)	0.297
Gestation (weeks)	29.2 (1.1)	26.6 (1.6)	<0.001
Birth weight (grams)	1160 (261)	924 (205)	0.007
Days on ventilator	1 (0-2)	6 (2-20)	<0.001
Days on CPAP	10 (6-27)	30 (14-39)	0.028
Days on supplementary O2	28 (7-51)	50 (21-73)	0.051

Values are mean (SD), median (IQR) or n(%) as appropriate. *Retinopathy of Prematurity; † Red blood cell

Table 2: Comparison of haematological values between those infants who developed ROP and those that did not

	No ROP (95% CI) (n=22)	ROP (95% CI) (n=14)	p value
Initial Hb (g/L)	162.5 (153.2, 171.8)	143.6 (133.3, 153.9)	0.009
Mean Hb (g/L)	134.9 (116.0, 153.7)	112.4 (102.8, 122.1)	0.06
Initial %HbF	92.3 (89.9, 94.7)	83.3 (71.1, 95.5)	0.06
Mean %HbF	91.87 (87.2, 96.5)	61.75 (44.5, 79.0)	0.0001

Table 3: Association between haematological values and ROP

Variable	OR (95% CI)	Adjusted* OR (95% CI)
Initial HbF%	0.96 (0.93-1.00)	0.97 (0.91-1.03)
Mean HbF%	0.94 (0.90-0.97)	0.94 (0.90-0.99)

* Adjusted for birthweight, gestation and total transfusion volume. OR= odds ratio. CI= confidence interval.